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TITLE: Biomarkers of Renal Tumor Burden and Progression in TSC

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14. ABSTRACT
Renal lesions occur commonly in people with TSC and can cause significant morbidity and mortality. Although most solid renal lesions of TSC are benign angiomyolipoma (AML), some are in fact cancerous. Moreover, rapidly growing AMLs can be life threatening when abnormal blood vessels rupture. Based on the hypothesis that renal tumor growth is associated with measurable changes in urine composition and on the knowledge that angiogenesis is essential for tumor expansion, we have predicted that factors associated with angiogenesis and with renal injury will increase during periods of TSC-associated renal tumor growth. We are testing this candidate driven approach to identify factors in urine or serum that reflect renal tumor burden and whose concentrations change as tumors grow. This project can be described in two aims. (1) Monitor the progression of TSC renal disease by combining prospective data collection with retrospective chart review of a large population of TSC patients with respect to gender, age, patient size, TSC genotype, renal lesions (e.g. lesion number, appearance and growth rates) and renal function parameters (e.g. blood pressure, serum chemistries, urinalysis and urine chemistries). (2) Measure soluble growth factors, angiogenesis factors and renal injury molecules in urine and serum samples from patients with TSC and evaluate these candidates as surrogate markers of renal tumor burden and growth.

15. SUBJECT TERMS
renal angiomyolipoma; urine biomarkers

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Introduction:
Renal lesions occur commonly in people with TSC and can cause significant morbidity and mortality [1]. Although most solid renal lesions of TSC are benign angiomyolipoma (AML), some are in fact cancerous. Moreover, rapidly growing AMLs can be life threatening when abnormal blood vessels rupture [2]. Based on the hypothesis that renal tumor growth is associated with measurable changes in urine composition and on the knowledge that angiogenesis is essential for tumor expansion [3, 4], we have predicted that factors associated with angiogenesis and with renal injury will increase during periods of TSC-associated renal tumor growth. We are testing this candidate driven approach to identify factors in urine or serum that reflect renal tumor burden and whose concentrations change as tumors grow. This project can be described in two aims. (1) Monitor the progression of TSC renal disease by combining prospective data collection with retrospective chart review of a large population of TSC patients with respect to gender, age, patient size, TSC genotype, renal lesions (e.g. lesion number, appearance and growth rates) and renal function parameters (e.g. blood pressure, serum chemistries, urinalysis and urine chemistries). (2) Measure soluble growth factors, angiogenesis factors and renal injury molecules in urine and serum samples from patients with TSC and evaluate these candidates as surrogate markers of renal tumor burden and growth.

Body:
Even though this grant was awarded as of 09/01/2010, permission from the DoD to enroll human subjects was delayed until 08/04/2011. As a result of this nearly 12 month lag time and with the Scientific Officer's knowledge, I started working on the project and began drawing on the awarded funds only as of 09/01/2011. Even though the original 2 year grant period is now approaching its end (09/01/2010-08/30/2012), I have only completed the first of the two planned years of research (09/01/2011-present). During this interval I have spent or committed approximately 92% of the year 1 budget. I am now filing my first annual report and I am requesting a 12 month No Cost Extension to use the unspent balance of the award as originally budgeted (plus the 8% carryover from year 1) to continue year 2 of the project during the upcoming 12 months from 09/01/0/2012 to 08/30/2013.

Point by point progress on this project is indicated here in prose and is appended in tabular form as a Statement of Work Update. It is important to note that this project period, as planned, has been exclusively one of patient enrollment and sample collection. Since no experiments have been performed as yet, there are therefore no data to report as yet.

The Statement of Work is divided into four blocks of 6 months each. Goals met during the first 6 months of the project, from 09/01/2011 to 03/01/2012, included initiation of the study with hiring and training of personnel, purchase of start-up equipment and education of support staff. Patient enrollment began smoothly in October 2011 so that 36 patients (target 50-100) were enrolled and 41 urine and blood samples (target 30-60) were collected, de-identified and processed for secure storage. The project database has been expanded and updated accordingly. Goals met during the second 6 months of the project, from 03/01/2012 to the present have included ongoing patient enrollment, sample collection and database expansion.

The total patient enrollment to date (N=71) has been slightly lower than anticipated (target 75-150). Three previously unappreciated factors have affected patient enrollment during TSC clinic

visits, as follows. (1) Not all cognitively impaired TSC patients are accompanied by their legal guardians. These patients are therefore not approached for the study in absence of a guardian. (2) TSC patients under stress that are seen urgently for acute issues rather than routine annual follow up have not been approached regarding the study. (3) Non-TSC patients with acute issues are periodically given appointment slots in TSC clinic, thus undermining our initial estimates that were based on total patient visits per TSC clinic per month rather than total TSC patient visits per TSC clinic per month. As a result of these observations, we are revising our projections for the upcoming 12 month project period. We anticipate that within the next year we will enroll 120-175 patients in all, rather than 150-200 (see updated SOW). Despite lower than anticipated patient enrollment, we have done quite well with sample collection. With an original target of 40-80 samples to be collected by the end of the first 12 months of the study, we have obtained 77 samples to date, which are slated for biomarker assays in year 2 of the project. We have therefore increased our estimates for second year sample collection accordingly, anticipating as many as 100-140 samples rather than the original projection of 60-100.

Interim analysis of the 71 patients enrolled to date reveals that 61% are female. Mean patient age at time of enrollment was 21 years, with a range from 2 to 66 years. Of the patients enrolled, 96% had TSC gene testing performed as part of their clinical care. TSC1 and TSC2 gene mutations were identified in 26% and 65% of the tested patients, respectively; 9% of tested patients had no mutations identified. Two of the patients with TSC2 gene mutations have TSC2/PKD1 contiguous gene syndrome with ADPKD in addition to TSC. Of the 67 patients with accessible renal imaging (*i.e.* not just reports from outside medical centers), 10% had no renal lesions, 87% had cysts and 70% had solid tumors. Distribution of renal lesions by TSC genotype is shown in Table 1. Only 2 patients had solid lesions in absence of cysts and one of those had clear cell RCC. The 90% incidence of renal lesions in this patient population is higher than previously reported, possibly due to an increased detection of small renal cysts through our routine use of MRI.

Table 1	TSC1 (N=16)	TSC2 (N=43)
cysts	63%	93%
masses	13%	88%
both	13%	84%
neither	38%	2%

Nearly half (47%) of patients with solid lesions (equivalent to 33% of all enrolled patients) underwent emergent or elective intervention in the form of biopsy, nephrectomy, embolization and/or pharmacotherapy with an mTOR inhibitor. None of these patients had TSC1 gene mutations. Our observation that one third of patients with TSC required some kind of intervention for renal lesions substantiates the need for an improved understanding of TSC associated renal lesions which will in turn drive the development of better diagnostics, prognostics and therapeutics.

Key Research Accomplishments:

The most significant accomplishment to date is the initial collection of a relatively large number of urine and blood samples from a carefully monitored population of people with TSC. This resource can potentially grow into a “tissue bank” for supplementary follow up studies beyond the scope of the current project.

Reportable Outcomes:

The existence of this DoD award enabled application for additional funds to supplement this project. As a result, the project has been awarded a $10K credit at RayBiotech, Inc., a commercial

company that produces reagents, kits and large scale protein arrays designed for biomarker discovery and validation studies. See appendix 2 for the notice of award.

Conclusions:
The Statement of Work indicates that year 1 of the project is predominantly patient enrollment and sample collection. Interim results are therefore limited to chart review observations in our patient population. We have noted that renal cysts are more common that previously reported, present in a majority of patients regardless of TSC genotype. In contrast, while a third of TSC patients require intervention for renal lesions, those that do are exclusively within the TSC2 and NMI patient subgroups. This observation supports the notion that TSC genotype has prognostic implications, which should in turn impact standard of care for clinical management of these patients.

References:

1. Rakowski, S.K., et al., *Renal manifestations of tuberous sclerosis complex: Incidence, prognosis, and predictive factors.* Kidney Int, 2006. **70**(10): p. 1777-82.
2. Shepherd, C.W., et al., *Causes of death in patients with tuberous sclerosis.* Mayo Clin Proc, 1991. **66**(8): p. 792-6.
3. Han, W.K., et al., *Urinary biomarkers in the early diagnosis of acute kidney injury.* Kidney Int, 2008. **73**(7): p. 863-9.
4. Naumov, G.N., L.A. Akslen, and J. Folkman, *Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch.* Cell Cycle, 2006. **5**(16): p. 1779-87.

Appendix 1 **Statement of Work:** Original projections are indicated black and white.
Current status and revised projections are indicated in yellow.

Project period (months):	**0-6**	**6-12**	**12-18**	**18-24**
Actual start date 09/01/2011	**09/01/2011-03/01/2012**	**03/01/2012-09/01/2012**	**09/01/2012-03/01/2013**	**03/01/2013-09/01/2013**
Task 1: initiate study				
1.a hire research personnel	X completed			
1.b train research personnel	X completed			
1.c purchase start-up equipment & supplies	X initiated			
1.d inform/ educate clinical assistants/ support staff	X initiated	X ongoing	X planned	X planned
Task 2: patient recruitment (at scheduled office visits)				
2.a prepare information packet	X completed			
2.b distribute information packet	X initiated	X ongoing	X planned	X planned
2.c obtain informed consent/ assent	X initiated	X ongoing	X planned	X planned
Milestone: projected number of patients enrolled	50-100	75-150	125-175	150-200
Actual number of patients enrolled (and revised projections)	36	71	(100-150)	(125-175)
Task 3: data collection and documentation				
3.a expand database record fields	X initiated			
3.b add new patients as needed	X initiated	X ongoing	X planned	X planned
3.c update database with office visit data *(e.g. height, weight, blood pressure)*	X initiated	X ongoing	X planned	X planned
3.d update database with clinical lab data *(e.g. serum chemistries, urinalysis)*	X initiated	X ongoing	X planned	X planned
3.e update database with radiologic data *(renal ultrasounds, CT scans, MRIs)*	X initiated	X ongoing	X planned	X planned

3.f update database with genetic data *(e.g. TSC1, TSC2 gene mutations)*	X initiated	X ongoing	X planned	X planned
3.g update database with event data *(hemorrhage, embolization, biopsy, nephrectomy)*	X initiated	X ongoing	X planned	X planned
Task 4: radiologic review				
4.a secondary review of radiology studies to confirm clinical reports	X initiated	X ongoing	X planned	X planned
4.b reconcile differences between primary and secondary review	X initiated	X ongoing	X planned	X planned
4.c correct database entries as needed	X initiated	X ongoing	X planned	X planned
Task 5: fluid collection				
5.a collect blood samples, de-identify, prepare for long term storage	X initiated	X ongoing	X planned	X planned
5.b collect urine samples, de-identify, prepare for long term storage	X initiated	X ongoing	X planned	X planned
Milestone: projected number of samples collected	30-60	40-80	50-90	60-100
Actual number of samples collected (and revised projections)	41	77	(90-120)	(100-140)
Task 6: biomarker screening				
6.a assign patient groups by genotype and phenotype	X initiated	X ongoing	X planned	X planned
6.b select patients for initial screening		X completed		
6.c purchase materials / reagents for screening arrays		X initiated		
6.d perform urine screens			X planned	X planned
6.e perform blood screens			X planned	X planned
Milestone: projected number of samples screened			5-10	10-20
(revised projections)			(30)	(30)

6.f identify differentially expressed markers			X planned	X planned
Task 7: biomarker quantitation				
7.a purchase materials / reagents for quantitative immunoassays			X planned	
7.b optimize quantitative protocols			X planned	 planned
7.c quantify relevant blood biomarkers			X planned	X planned
7.d quantify relevant urine biomarkers			X planned	X planned
Milestone: projected number of samples quantified per biomarker (revised projections)			40-80 (70-100)	60-100 (90-120)
Task 8: data analysis				
8.a re-assign patient groups as needed *(based on interim disease progression)*			X planned	X planned
8.b data tabulation and statistical analysis				X planned
8.c manuscript preparation				X planned

Appendix 2

From: Brett Burkholder <brett@raybiotech.com>
Date: Mon, 10 Oct 2011 19:01:09 -0400
To: Elahna Paul <epaul@partners.org>
Subject: Official Notice of Grant Award

Dear Dr. Paul,

Congratulations! You have been selected as an Awardee of a 2011 Biomarker Discovery Pilot Grant from RayBiotech in the amount of **$10,000**. This was a competitive grant process comparing applications from all over the world. At least 4 reviewers scored your submission, and less than 10% of all applicants were chosen for such an award, so you should be very proud of this accomplishment.

To redeem this Biomarker Grant Award on any purchase order, please send your request directly to RayBiotech USA (by fax at +1-770-206-2393 or by email to orders@raybiotech.com) and reference the following Grant Award Number on your purchase order: **RBG2011-PAUL-10K**.

Again, congratulations on your Biomarker Discovery Pilot Grant award. We look forward to working with you and wish you the best of success in your research.

Kind regards,

Brett Burkholder
Mgr., Marketing and Business Development

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